The 12/8/99 HIARC report represents the latest toxicological information and the most recently selected endpoints for the vinclozolin risk assessments. The last toxicology chapter for the vinclozolin RED was written by HED in 1997; this chapter was not included in the docket because it is outdated. Readers should refer to the HIARC report for the most recent toxicology information for vinclozolin.

HED DOC. NO. 013919

DATE: **December 8, 1999**

MEMORANDUM

SUBJECT: ***VINCLOZOLIN***: - 2nd Report of the Hazard Identification Assessment Review Committee.

FROM: David G Anderson and Elizabeth Mendez
Toxicologist, RRB-2 Toxicologist, RRB-1
Health Effects Division (7509C) Health Effects Division (7509C)

THROUGH: Pauline Wagner, Co-Chairperson
And
Jess Rowland, Co-Chairperson
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: William Hazel, Risk Assessor
Reregistration Branch-1
Health Effects Division (7509C)

PC Code: 113201

On September 16, 1999, and on November 4, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of Vinclozolin and re-assessed the existing developmental endpoint for acute dietary as well as occupational and residential exposure risk assessments. HIARC also addressed the potential enhanced sensitivity to infants and children as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report. The September 16, 1999 meeting considered additional data on the ventral prostate weight in male offspring and literature data on potential neurotoxic effects from perinatal testing. The potential neurotoxic effects found in the literature needed a more thorough review and an additional meeting of the HIARC was scheduled for November 4, 1999 to review this data in detail. These two meetings are reported together, since the November 4, 1999 meeting is a combination of the September 16, 1999 meeting.

Committee Members in Attendance at the 9/16/99 meeting

Members present were David G Anderson, William Burnam, Virginia Dobzoy, Karen Hamernik, Pam Hurley, Mike Iannaou, Tina Levine, Susan Makris, Nicole Paquette, Kathy Raffaele, PV Shah, Jess Rowland (CoChair) and Pauline Wagner (CoChair). Members in absentia were Nancy McCarroll and Brenda Tarplee (Exec. Sec.). Data was presented by David G Anderson of Re-Registration Branch 2.

Other HED members present were William Hazel, and Mike Metzger. Deanna Scher from SRRD was present.

Committee Members in Attendance at the 11/4/99 meeting

Members present were David G Anderson, William Burnam, Pamela Hurley, Mike Iannaou, Tina Levine, Susan Makris, Nicole Paquette, Kathy Raffaele, PV Shah, Jess Rowland (CoChair), Pauline Wagner (CoChair), and Brenda Tarplee (Exec. Sec.). Members in absentia were Nancy McCarroll, Virginia Dobozy, and Karen Hamernik. Data was presented by Elizabeth Mendez of Reregistration Branch-1.

Other HED members present were Whang Phang, Mike Metzger, Anna Bearden Lowit, G Jeffrey Herndon. Deanna Sher and John Leahy of SRRD were also present.

Data Presentation and
Report Preparation: _____

David G Anderson, Ph.D, Toxicologist

and

_____

Elizabeth Mendez, PhD, Toxicologist

1.0 INTRODUCTION

On August 5, 1995, the Health Effects Division's (HED's) RfD/Peer Review Committee established a Reference Dose (RfD) of 0.012 mg/kg/day based on a NOAEL of 1.2 mg/kg/day and an Uncertainty Factor of 100 for inter-species extrapolation and intra-species variability (*Memorandum:* G. Ghali, HED to S. Lewis, RD, dated January 30, 1996).

On August 15, 1995 the Health Effects Division's Toxicology Endpoint Selection (TES) Committee selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments. An *ad hoc* meeting held on April 17, 1997, addressed the potential problems in assessing hazards associated with treatment of recreational areas, lawns and other home uses of Vinclozolin. Doses and endpoints were selected for Short-and Intermediate-Term residential exposures for the subpopulation (Infants and Children) (TES Document-revised dated June 2, 1997).

The HED's Reproductive and Developmental Toxicity Assessment Review Committee met on October 20, 1993, January 23, 1997 and December 3, 1997 to evaluate the weight-of-the evidence for reproductive and developmental toxicity of Vinclozolin .

At the October 20, 1993 meeting, the Committee concluded that the critical effect level for risk assessments for developmental toxicity was 3 mg/kg/day (lowest dose tested) based upon decreased ano-genital distance in males in a rat developmental study (Gray study, 1993, MRID 43170501).

At the January 23, 1997 meeting, held to evaluate the Uncertainty Factor, the *ad hoc* Committee decided that an Uncertainty Factor of 100 was appropriate. The December 3, 1997 meeting, was held to determine a suitable endpoint for the regulation of potential developmental effects of Vinclozolin because new material had become available. The Committee concluded that for developmental toxicity, the NOAEL was 6 mg/kg/day and the LOAEL was 12 mg/kg/day based on statistically significant decreases in male ano-genital distance (Gray, 1993) and increased hairless areas, reported as areolas (BASF studies). (*Memorandum:* K. Farwell to C. Swentzel, dated January 28, 1998).

On February 5, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) re-assessed the existing RfD, the toxicology endpoints selected for dietary and occupational/ residential exposure risk assessments, and addressed the potential susceptibility of infants and children to exposure to Vinclozolin pursuant to the Food Quality Protection Act (FQPA) of 1996. The application of the FQPA safety factor to ensure for the protection of Infants and Children to Vinclozolin, as required by FQPA, will be determined during risk characterization.

In March of 1999, additional data on prostate weight in male offspring resulting from perinatal studies became available. In addition, other literature became available on potential effects of vinclozolin on the brain. These data were re-evaluated in a HIARC meeting on September 16.

On September 16, 1999, the HIARC considered the additional data on ventral prostate weight in male offspring and required additional evaluation of open literature on potential brain effects from antiandrogens. On November 4, 1999, the HIARC re-evaluated the literature data on potential effects on the brain and other potential neurotoxic effects from perinatal testing of substances that have androgenic and antiandrogenic effects on exposed offspring. The September 16 and the November 4, 1999 meeting conclusions are combined in the

current report.

This report supersedes the previous HIARC, RfD and TES Committee reports.

2.0 HAZARD IDENTIFICATION

2.1.0 DIETARY

2.1.1 ACUTE DIETARY (Acute RfD for females 13+)*

Type of Study Proposed: **Guideline #:** NG

A perinatal developmental study by Gray et al., 1999; Prenatal Developmental Toxicity - Rat (Data from a 1996 SAP presentation) and two studies by BASF (MRID# 44395701 and 44395702)

MRID No.: Literature reference only: Gray Jr, EL, Ostby, J, Monosson, E, and Kelce, WR Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. Toxicology and Industrial Health 15: 48-64 (1999).

Executive Summary:

Gray et al., 1999 reported that there was no threshold for effects on AGD, areolas/nipple development and prostate weight decrement and believe that the lowest dose level tested was an effect level (although they admit that the prostate weight decrement at the lowest dose level is not statistically significant). Their paper uses several graphics for support of a no threshold for these antiandrogenic effects.

[A model has been postulated, which supports the lack of a threshold for hormone modulation of endogenous hormones, such as testosterone and estrogen. However, this belief is controversial among qualified scientists and no definitive Agency or OPP policy on the subject has been made at this time.]

Executive Summary (Prepared by D Anderson for Gray et al., 1999): Vinclozolin (>99% purity) was administered by gavage in corn oil vehicle (2.5 ml/kg) to 4 blocks of rats at 0, 3.125, 6.25, 12.5, 25, 50 or 100 mg/kg/day from gestational day (GD) 14 through postnatal day (PND) 2-3 (not all dose levels were used in all Blocks). Ano-genital distance (AGD) was measured on live pups at PND 2, 8, 15, and 22 (all 4 Blocks). At PND 12 - 14, the pups were examined for areolas (hairless patches) and nipple development (buds) (Blocks 1 and 4 reported). At PND day 55-56, 40 surplus male offspring (1-3 per litter) from Block 1 were necropsied: with 9 pups (from 5 litter), 9 pups (3 litters), 6 pups (3 litters, 6 pups (2 litters, and 5 pups (2 litters) treated at 0, 3.125, 6.26, 12.5, 50 , and 100 mg/kg/day, respectively. Body weights and reproductive organs weights were taken. The remaining male rats were necropsied and examined at 12 months. Blocks 2 and 3 were similarly examined for AGD and areolas/nipple development. AGD and areolas/nipple development were examined in Block 4 (similarly to Block 1, 2, and 3), and reproductive organ weights were examined at 14 months in Block 4.

Since dosing was stopped on PND 3, all effects demonstrated can be classified as developmental effects. **Table 1** summarizes the new data on prostate weight, nipple and areolas development, which are the focus of our attention. The data were extracted from Gray et al. (1999).

The data show that there was a dose related decrease in prostate weight in male offspring at 6.25 mg/kg/day and above. The failure to show a statistically significant decrement in prostate weight in male offspring at 12.5 mg/kg/day was possibly due the small sample size and the difficulty in accurately determining ventral prostate weight. The prostate weight show a NOAEL/LOAEL of 3.125/6.25 mg/kg/day.

The data show that areolas formation in male offspring was dose related and statistically significant at

3.125 mg/kg/day and above (**Table 2**). Gray claimed that the 3.125 mg/kg/day dose level also showed statistically significant increased areolas formation, but in the SAP report, Gray did not claim statistical significance for the same data values at 3.125 mg/kg/day.

The data show that a nipple developed in 1 pup from a dam dosed at 3.125 mg/kg/day and in increasing percentages up to 50 mg/kg/day where 100% nipple development occurred in male offspring. Although only the 50 mg/kg/day dose level showed statistically significant increases, historical control data in their laboratory showed no spontaneous nipple development in over 200-300 male offspring examined. (In addition, since the uterine position of some males must have been between two females among the 200-300 control male pups examined, the significance in one exposed male is increased.) Under these circumstances, nipple development in male offspring would be classified as a rare event and a single nipple development in a single pup may be a biologically significant event. Consequently, Gray considered the NOAEL/LOAEL for nipple development is ---/3.125 mg/kg/day. The HIARC examined this data, but did not accept the argument. Since histological confirmation was conducted only for the nipples at the 12.5 dose level, the data on the nipple development at lower dose levels was in doubt.

OPP does not have individual animal data nor has it validated the statistical analyses conducted on any of these parameters except the AGD.

Prenatal Developmental Toxicity - Rat (Data from Gray's 1996 SAP presentation)

The report included results from other studies by the author showing increased serum luteinizing hormone at 5 mg/kg/day and above from vinclozolin administration from weaning to day 100; delayed puberty at 15 mg/kg/day and above, and at 100 mg/kg/day and above, bladder stones, increased mortality, seminiferous tubular atrophy, testicular and epididymal granulomas, prostate agenesis, and increased serum testosterone.

Other Evidence on AGD and Areola/Nipple Incidence from BASF

MRID# 44395701. Hellwig, J. (1997 a). Reg No. 83 258 - Pre-/postnatal toxicity Study in Wistar Rats after Oral Administration (Gavage). Dept of Toxicology BASF Akiengesellschaft, FRG. 9/1/97. Unpublished.

MRID# 44395702. Hellwig, J. (1997 b) Reg No. 83 258 - Pre-/postnatal toxicity Study in Long Evans Rats after Oral Administration (Gavage) Dept of Toxicology BASF Akiengesellschaft, FRG. 9/1/97. Unpublished.

The registrant (BASF) sponsored developmental studies in Long-Evans and Wistar following Dr. Gray's protocol, including postnatal dosing to include the period of sexual development (gestational day (GD) 14 to postnatal day (PND) 2-3). A similar vehicle (olive oil compared to corn oil) was used. The 2 studies were an attempt to duplicate Dr. Gray's report of decreased ano-genital distance (AGD) in LE rats at the low dose of 3 mg/kg/day.

Vinclozolin was administered to 20 pregnant LE rats per group and 20 pregnant Wistar rats per group by gavage in olive oil from gestation day 14 to postnatal day 3 at doses of 0, 1, 3, 6, 12, or 200 mg/kg/day in LE rats and 0, 3, 12, or 200 mg/kg/day in Wistar rats. AGD was measured on live male pups on day 2, and day 22 as in the Gray study. About half the male pups were then sacrificed, fixed in Bouin's solution and the AGD remeasured. The study was conducted in 2 blocks separated by 1 day. The results are shown in **Tables 3 and 4** .

The only biologically significant maternal effect was decreased weight gain in Wistar rats (-90%), accompanied by decreased food consumption in the 200 mg/kg/day group. For maternal toxicity, the NOAEL

was 12 mg/kg/day and the LOAEL was 200 mg/kg/day based on decreased weight gain and food consumption in LE rats.

The AGD or AGD-index were statistically significantly reduced only at 200 mg/kg/day in fixed and live pups from each strain. In addition, all the male pups in both strains had ambiguous genitalia at the 200 mg/kg/day dose level only. Also in both strains at 200 mg/kg/day, live birth index was decreased (Wistar & LE: 88%-83% versus 98%-93% in controls, respectively) and number of stillborn were increased (Wistar & LE: 12% & 17% versus 2.2% & 6.8% in controls, respectively) as well as pups that died (38% & 15% versus 1.5% & 8.1% in controls, respectively) and decreased survival day 0 to 2 (58% & 65% versus 98% & 92% in controls, respectively). Day 2 pup weights were reduced (25%, male, and 27%, female, of control in Wistar rats) and at day 22 they were reduced (14% of control, p#0.05 in Wistar female rats), but only nominally reduced from control in Long Evans rats at day 2 and day 22 at 200 mg/kg/day. The areolas/nipple anlagen was presented on a litter basis (statistical analyses not conducted) and % pup basis (p#0.05) at 200 and 12 mg/kg/day in both strains (48.1% versus 11.3% in control in Wistar pups and 61.3% versus 31.1% in control in Long Evans pups). For overall developmental toxicity, the NOAEL/LOAEL was 6/12 mg/kg/day based on significant increase in areolas/nipple anlagen in both strains. For AGD, the NOAEL/LOAEL was 12/200 mg/kg/day based on statistically significantly decreased ano-genital distance. For nipples, the NOAEL/LOAEL was 12/200 mg/kg/day but for areolas the NOAEL/LOAEL was 6/12 mg/kg/day in Wistar rats. For nipples and areolas development in LE rats, the NOAEL/LOAEL was 6/12 mg/kg/day.

Table 1: Summary of effects of vinclozolin (administered GD 14 to PND 3) on body and organ weights and other parameters measured in LE male rat offspring about 12 months of age (Block 1 and 4).[a] (Information and data extracted from Gray et al (1999)

Dose levels (mg/kg/day)	0	3.125	6.25	12.5	25	50	100
Body Wt (g), data±SE (#litters/#males)	709±38 (9/30)	681±20 (7/37)	691±17 (8/39)	726±6 (7/46)	695±5 (4/18)	716±36 (3/6)	644±32 (2/2)
Seminal vesicle wt (mg) [b] (#litters/#males)	1945±147 (9/27)	1883±147 (7/36)	1747±102 (8/37)	1800±100 (7/45)	1744±134 (4/18)	1859±126 (3/5)	657±142 [c] (2/2)
Ventral prostate wt. (mg)[b] (#litters/#males)	**564±57 (9/27)**	**499±50 (7/36)**	***415±40 (8/37)**	**439±31 (7/45)**	****382±27 (4/18)**	****305±33 (3/5)**	****69±47 [c] (2/2)**
Testes wt (g) (#litters/#males)	3.71±0.09 (9/24)	3.63±0.06 (7/19)	3.55±0.12 (8/23)	3.69±0.15 (7/18)	3.82±0.14 (4/13)	3.55±0.12 (3/6)	3.48±0.14 (2/2)
Adrenal wt (mg) (#litters/#males)	46±3 (5/13)	50±6 (3/12)	49±1 3/13	43±5 (2/10)	43±0.2 (2/6)	45±3 (3/6)	60±19 (2/2)
Cauda epididymides (mg) (#litters/#males)	316±9 (9/24)	311±10 (7/19)	303±9 (8/23)	323±7 (7/19)	304±16 (4/13)	287±21 (3/6)	**213±82 (2/2)
Cauda epididymal sperm (x 10^6) (#litters/#males)	152±9 (5/12)	134±13 (3/12)	133±16 (3/13)	152±10 (2/10)	153±20 (2/6)	148±25 (3/6)	*67±67 (2/2)
Testis spermatids (x 10^6) (#litters/#males)	218±11 (5/13)	213±4 (3/12)	206±4 (3/13)	219± (2/10)	223±22 (2/9)	191±28 (3/6)	175±7.5 (2/2)
Low ejaculated sperm count (<10^6)	0/14	0/13	1/13	0/10	0/6	3/6	3/3
Ejaculated sperm (x 10^8)	1.84±0.12 (5/13)	1.87±0.17 (3/12)	1.82±0.15 (3/12)	2.03±0.1 (2/10)	1.93±0.01 (2/6)	**0.18±0.16 (3/6)	**0 (3/3)
Fertility	100%	100%	92%	100%	100%	*50%	**0%
Percentage with hypospadia (#litters examined/#males examined)	0 (19/77)	0 (16/96)	0 19/117)	0 (12/84)	0 (11/56)	45±16 (3/11)	100 (2/7)
Percentage with nipples (#litters examined/#males examined)	**0 (19/77)**	**1.0±1.0 (16/96)**	**2.6±1.4 (19/117)**	**3.6±2.0 (12/84)**	**5.4±3.0 (11/56)**	****91.0±9.0 (3/7)**	****100 (2/7**
(# pups with nipples)/(#pups examined); [minium # litters affected]	**0/200-300 [d] [0]**	**1/96 [1]**	**3/117 [2 or 3]**	**3/84 [2 or 3]**	**3/56 [2 or 3]**	**6/7? [3]**	**7/7 [2]**
Serum testosterone (ng/ml)	1.85±0.55 (5/13)	1.35±0.14 (3/12)	1.70±0.37 (3/13)	1.94±0.2 (2/10)	1.89±0.05 (2/6)	1.39±0.53 (3/6)	1.52±0.41 (2/2)
Percentage ectopic testes	0 19/77)	0 (16/96)	0 (19/117)	0 (12/84)	0 (11/56)	0 (3/11)	20 (2/10)

Bolded rows are effects under consideration. [a] = Data was analyzed using litter means. [b] = Seminal vesicle and prostate weights were not measured in those males that displayed gross inflamation and/or discoloration of the sex accessory tissue. [c] = Tissue were inflamed but included for comparison [d] = Historically, no nipples have been detected in 200-300 control LE male pups. [e] = Minimum litters affected was calculated by the reviewer from the % pups affected and with reference to number affected in Block 1.

Table 2: Summary data on LE male offspring from Gray et al. (1996 and 1999) (Table extracted from the RDARC of 1998)

Dose levels (mg/kg/day)	0	3.125	6.25	12.5	25	50
% nipple development in 13 day old pups examined, reported for Block 1 & 4, only (# male pups affected/# pups examined)	0 (0/200)	1.0 (1/96)	2.6 (3/117)	3.6 (3/84)	5.4 (3/56)	91 (7/7)
Areolas development in 13 day old pups Percentages are from all 4 Blocks (# litter examined/# pups examined)	4.9% (20/112)	17.4%[a] (18/122)	*33.1% (19/135)	*55.3% (13/99)	*49% (12/82)	*100% (2/12)
Block 1 AGD in 2-day old LE pups (#litters)	3.40 (5)	3.23 (3)	3.24 (3)	2.98 (3)	2.85 (2)	2.55 (2)
Block 2 AGD in 2-DAY OLD LE pups (#litters)	3.09 (5)	2.92 (5)	2.89 (5)	---	--	---
Block 3 AGD in 2-DAY OLD LE pups (#litters examined)	3.61 (6)	3.12 (6)	3.50 (6)	3.17 (5)	3.12 (7)	---
Block 4 AGD in 2-DAY OLD LE pups (# litters examined)	3.59 (4)	3.58 (4)	3.49 (5)	3.45 (5)	2.99 (3)	---
Blocks 1-4 AGD. 2-DAY OLD LE pups (total # litters examined/# males)	3.43 (20/112)	*3.18 (18/122)	*3.29 (19/135)	***3.23** (13/99)	***3.05** (12/82)	***2.55** (2/12)

[a] = Stated to be statistically significant at $p < 0.05$ by a one tailed T test by Gray et al. (1999). **Bolded values** were supportable as being statistically significant according to OPP. * = Claimed to be statistically significant by Gray et al. (1996 & 1999).

Table 3: Selected results from BASF vinclozolin study conducted by the Gray protocol in Long-Evans rats (Data extracted from MRID# 44404601 and page 18 of the RDARC of 4/7/98

	DOSE (mg/kg/day)					
	0	**1**	**3**	**6**	**12**	**200**
Areolas on males at day 12 (no histology performed)	31.1%	36.4%	44.5%	35.7%	**61.3%***	**100.0%***
Nipples on males at PND 60 (# pups affected/# examined)	0	0	0	0	6% (3/50)	**100%****
AGD day 2 live pups	3.72	3.86	3.67	3.54	3.70	**2.48****
AGD day 2, Bouin fixed pups	3.13	3.09	2.97	2.99	3.03	**1.74****
AGD day 22, live pups	15.69	15.42	15.01	14.95	14.63	**11.70****
AG Index day 22, live pups	0.26	0.26	0.28	0.27	0.27	**0.20***

* = Statistically significant at $p<0.05$ according to BASF

Table 4: Selected results from a BASF vinclozolin study conducted by the Gray protocol in WISTAR rats (Data extracted from MRID# 44404601 and page 18 of the RDARC of 4/7/98)

	DOSE (mg/kg/day)					
	0	**1**	**3**	**6**	**12**	**200**
% males with areolas day 12 (no histology performed)	11.3	---	8.3	---	**48.1***	**100.0****
Nipples at PND 60	0	0	0	0	0	Acknowledged to be high
AGD day 2, live pups	3.93	---	4.20	---	3.94	**2.07****
AGD day 2, Bouin fixed pups	3.21	---	3.31	---	3.25	**1.68****
AGD day 22 live pups	16.89	---	16.34	---	17.11	**10.93****
AG Index day 2 live pups	0.51	---	0.51	---	0.49	**0.36****
AG Index day 2, Bouin fixed pups	0.41	---	0.41	---	0.40	**0.30****
AG Index day 22, live pups	0.27	---	0.27	---	0.26	**0.20****

***** p#0.05 ****** p#0.01

Dose and Endpoint for Risk Assessment: Adjusted Dose= 6 mg/kg/day. This adjusted dose was derived by the application of a plasma equilibrium factor of 1.84 to the NOAEL of 3.125 mg/kg/day. (i.e., NOAEL 3.125 mg/kg/day x 1.84=5.75 mg/kg/day and rounded off to 6 mg/kg/day).

Comments about Study and Endpoint: A plasma equilibrium factor was used since the dose that would have induced the developmental effects would have been higher because the acute effect level (6.25 mg/kg/day) could not be attained with a single dose of 6.25 mg/kg/day. The plasma equilibrium factor was based on the peak plasma levels reached 2 hours after a single dose and 2 hours after the last of 7 daily doses in female rats in a metabolism study (MRID Nos. 41824307/ 41824308). Following a single oral dose at 10 mg/kg, the peak plasma level at 2 hours post dosing was 2.12 ：g/g-plasma whereas following 7 daily doses at 10 mg/kg, the peak plasma level 2 hours after the last dose was 3.91 ：g/g-plasma indicating that the plasma levels would be close to equilibrium levels in rats after 7 days of dosing. Therefore, the ratio (plasma equilibrium factor) is 1.84 (i.e., 3.91 ：g/g÷2.12 ：g/g = 1.84).

In addition, it is reasonable to further assume that the maximal developmental effects occur from the peak plasma levels of Vinclozolin. However, it is unknown when the peak plasma levels of the antiandrogenic metabolites would occur, but presumably these would be no higher than plasma levels of the parent compound, Vinclozolin. **Therefore, an adjusted dose of 6 mg/kg/day was calculated only for acute dietary risk assessment (data rounded off).**

The HIARC based the endpoints on the results of the three studies presented and

determined that for developmental toxicity,

- The NOAEL was 3.125 mg/kg/day (unadjusted for a single dose) and the LOAEL was 6.25 mg/kg/day (unadjusted for a single dose). The LOAEL is based on statistically significant decreases in ventral prostate weight in male offspring (Gray et al. 1999).
- The acute NOAEL level was changed from 6.25 mg/kg/day (unadjusted for a single dose) to 11.5 mg/kg/day (adjusted for a single dose) in the previous HIARC of March 15, 1998, to 3.125 mg/kg/day (unadjusted for a single dose) to 6 mg/kg/day (adjusted for a single dose) in the current HIARC report because the additional data by Gray et al. 1999 suggested the lower NOAEL was more appropriately based on the ventral prostate weight decrement.

The areolas/nipple development endpoints at 3.125 mg/kg/day were considered to be less reliable and at variance with the data generated by BASF on increased hairless areas, reported as areolas/nipple anlagen in Long Evans and Wistar strains (Hellwig, 1997 a,b). This decision was based on low confidence in the effects seen at 3.125 mg/kg/day and the following factors:

- The single nipple in 1 rat pup was insufficient to determine an adverse effect.
- There was no evidence that this single nipple at 3.125 mg/kg/day was confirmed histologically.
- The prostate weight in male offspring at 3.125 mg/kg/day were not statistically significantly different from controls values.
- The ambiguous nature of areolas identification even though the incidence was statistically significantly different from controls values.
- The BASF study showed a higher background incidence and showed a statistically significant difference from control values at a higher dose level.
- The decreased (AGD) in male rats (Gray , 1993) was statistically significant at 12.5 mg/kg/day and above by multiple statistical analyses by the Science Analysis Branch of HED (*Memorandum:* M. Marion to D. Anderson, dated 11/19/97).
- The decreased AGD by itself would be of uncertain concern for human risk assessment since this effect occurs in rats but the relevance to humans is unknown. However, when taken into consideration with other effects occurring at higher dose levels, it provides a sensitive indicator for anti-androgenic effects.
- There was wide variation for ano-genital distances among the different control groups in the study conducted by Gray, 1993.
- Decreased ano-genital distance did not occur at 12 mg/kg/day in the two studies by the Registrant (Hellwig, 1997 a,b).

Uncertainty Factor (UF): 10x for inter-species extrapolation, 10x for intra-species variability. Total UF is 100.

ACUTE RfD = 6 mg/kg/day (Adjusted NOAEL to an acute dose) = 0.06 mg/kg/day
100 (UF)

This risk assessment is required.

2.1.2 Acute Dietary General Population including Infants and Children

There were no toxicological effects applicable to these populations and attributable to a single exposure (dose) observed in oral toxicity studies including the developmental toxicity studies in mice, rats and rabbits. Therefore, a dose and endpoint was not identified, and an acute dietary risk assessment is not required for these subpopulations,

This risk assessment is **NOT** required.

2.2 Chronic Dietary [Reference Dose (RfD)]

The RfD established in 1995 was re-assessed by this Committee pursuant to the FQPA and is discussed below:

Studies Selected:	Chronic Toxicity - Rat	§83-1a
	Combined Chronic Toxicity/Carcinogenicity - Rat	§83-5
MRID Nos.	43254701, 43254702 and 43254703	

Executive Summaries: In the chronic toxicity study (MRID No. 43254701), a NOAEL was not established. The lowest dose tested 150 ppm (7 mg/kg/day in males and 9 mg/kg/day in females) was the LOAEL and was based on bilateral lenticular degeneration of the eyes, tubular calcification in the testes and interstitial fibrosis of the prostate and interstitial cell lipidosis of the ovaries in females.

In the second chronic toxicity study (MRID No. 43254702), groups of 20 male and 20 female Wistar rats were fed diets containing Vinclozolin at 0, 25 or 50 ppm (equivalent to 0, 1.2, or 2.4 mg/kg/day for males and 0, 1.6, or 3.2 mg/kg/day for females) for 104 weeks. The NOAEL was 50 ppm (HDT); a LOAEL was not established.

In the third study (MRID No. 43254703), groups of 50 male and 50 female Wistar rats were fed diets containing Vinclozolin at 0, 50, 500 or 3000 ppm (equivalent to 0, 2.3, 23, or 143

mg/kg/day for males and 0, 3, 30, or 180 mg/kg/day for females) for 104 weeks. A NOAEL was not established and the LOAEL was 2.3 mg/kg/day, the lowest dose tested.

Dose/Endpoint for establishing the RfD: NOAEL=1.2 mg/kg/day established based on the results of the three studies. The LOAEL was 2.3 mg/kg/day and was based on foam cell aggregates in the lungs (males), eosinophilic foci in the liver (males), interstitial cell lipidosis in the ovaries (females) and lenticular degeneration of the eyes (both sexes)

Comments about Study and Endpoint: Vinclozolin (metabolites/degeneration products) competitively inhibit the androgen receptor and has antiandrogenic activity. In addition, Vinclozolin interferes with lipid metabolism and/or storage. Other effects observed at one or mores doses included: lesions such as edema; diffuse tubular atrophy; tubular calcification; cystic rete testis and hyperplastic rete testis; azoospermia and oligospermia in the epididymides; degenerative lesions of the accessory organs; interstitial lipidosis of the ovary and adrenal glands; liver cell hypertrophy; lenticular calcification; vacuolated acinar cells of the pancreas; and focal fiber atrophy of the skeletal muscle. The HIARC re-affirmed the dose and endpoints selected by the RfD/Peer Review Committee.

Uncertainty Factor (UF): 10x for inter-species extrapolation, 10x for intra-species variability. Total UF is 100.

$$\text{Chronic RfD} = \frac{1.2 \text{ mg/kg/day (NOAEL)}}{100 \text{ (UF)}} = 0.012 \text{ mg/kg/day}$$

This risk assessment is required

2.3 Occupational/Residential Exposure

Dose and endpoints were also selected for the subpopulation Infants and Children because of the potential for Short-and Intermediate-Term exposures to Vinclozolin based on the use pattern (i.e., recreational areas, lawns and other home uses).

2.3.1 Dermal Absorption

The percentage dermal absorption and skin retention at various dermal doses of Vinclozolin at 10 hours after a single application are presented in the table below. Data taken from a dermal absorption study of vinclozolin on rats (MRID# 41824309)

Dose level (mg/kg)	0.13	1.3	13	130
Dose level (mg/cm^2)	0.002	0.02	0.2	2.0
Percentage Vinclozolin absorbed 10 hours after a single application (% of mg/kg)				
% absorbed through and retained in treated skin	**25.2**	19.5	6.16	4.42

Dermal Absorption Factor: 25.2% at 10 hours.

2.3.2 Short-Term Dermal - (1-7 days) Subpopulation: Female 13 +

Study Selected: Prenatal Developmental -Rat

MRID No. Gray, 1996 (None); 44395701 and 44395702

Executive Summary: See 2.1.1 Acute Dietary (Acute RfD for females 13+)

Dose and Endpoint for Risk Assessment: Developmental NOAEL= 3 mg/kg/day based upon statistically significant decreases in prostate weight in male offspring at 6.25 mg/kg/day.

Comments about Study and Endpoint: **No changes were made at this meeting.** The HIARC concurred with the TES Committee and the previous HIARC on the use of the oral developmental NOAEL for this risk assessment in spite of the availability of two dermal developmental toxicity studies (MRID Nos. 41413001 and 43703301). These studies are discussed in Section III. FQPA Considerations. In summary, groups of pregnant Wistar rats received repeated dermal application of Vinclozolin in 0.5% carboxymethylcellulose-water solution at doses of 0, 10, 20, 30 or 200 mg/kg/day (in the first study) and at doses of 0, 60, 180 or 360 mg/kg/day (in the second study), 6 hours/day during gestation days 6 through 20. Decreased AGD in male fetuses was observed at 180 mg/kg/day (LOAEL); the NOAEL was 60 mg/kg/day.

Although the Committee would have preferred to have used dermal studies if appropriate endpoints (e.g., ventral prostate weight in male offspring, the most sensitive endpoint) were measured in these studies, this was not the case. Therefore, the Committee selected the oral NOAEL for the following reasons:

(I) The most sensitive endpoint (decreased ventral prostate weight in male offspring) occurred at a lower dose via the oral (6.25 mg/kg/day) route in Long Evans Hooded rats, but ventral prostate weight was not measured for the dermal route in Wistar rats.

(ii) A comparison could not be made for the Long Evans Hooded rats since no dermal studies are available and thus the occurrence of this effect could not be substantiated in the Long Evans Hooded rats. Had the Registrant conducted the dermal developmental studies in Long Evans Hooded rats using the procedures similar to those of the Gray study for measuring ventral prostate weight, the Committee would have had more confidence in the dermal study.

Since an oral dose was selected, a dermal absorption factor of 25.2% should be used in dermal risk assessments.

This risk assessment is required.

2.3.3 Short-Term Dermal - (1-7 days) Subpopulation: Infants and Children

Study Selected: Postnatal Developmental -Rat

MRID No. None. Presentation by Dr. Gray to SAP on 10/39/96

Executive Summary: Long Evans Hooded rats received oral administration of Vinclozolin at dose levels of 0, 5, 15, 25 or 100 mg/kg/day from weaning to 15 weeks of age. A measure of puberty (age at preputial separation) was statistically significantly ($p < 0.05$) delayed (approximately 1 day) in males at 15 mg/kg/day (41.8 days), 25 mg/kg/day (44.4 days) and 100 mg/kg/day (47.8 days) when compared to males at 0 mg/kg/day (40.7 days). This finding was supported by significant decreases seen in caudal and paired epididymal weights at doses above 15 mg/kg/day. Although, the slightly delayed puberty is of unknown consequence it probably resulted from androgen deprivation from weaning to about day 40, during growth and development. The NOAEL was 5 mg/kg/day and the LOAEL was 15 mg/kg/day based on delayed puberty.

Dose and Endpoint for Risk Assessment: NOAEL= 5 mg/kg/day based on delayed puberty at 15 mg/kg/day(LOAEL).

Comments about Study and Endpoint/Uncertainty Factor: **No changes were made at**

this meeting. This endpoint is appropriate for use in risk assessment for this subpopulation (i.e., Infants and Children). This dose is supported by the NOAEL of 4.9 mg/kg/day established based on decrease in epididymal weights seen in male rats at 30 mg/kg/day in the two-generation reproduction study.

Since an oral dose was selected, a dermal absorption factor of 25.2% should be used in dermal risk assessments.

An Uncertainy Factor of 1000 was assessed for this endpoint (100 for intra and interspecies variation and the FQPA safety factor of 10X was retained for infants and children.

This risk assessment is required.

2.3.4 Intermediate-Term Dermal (7 Days to Several Months) Females 13 +

Study Selected: Prenatal Developmental -Rat

MRID No. Gray, 1996 (None); 44395701 and 44395702

Executive Summary: See 2.1.1 Acute Dietary, RfD for females 13+

Dose and Endpoint for Risk Assessment: Developmental NOAEL= 3 mg/kg/day based upon statistically significant decreases in prostate weight in male offspring with a LOAEL of 6.25 mg/kg/day and supported by increased hairless areas, reported as areolas/nipple anlagen in Long Evans and Wistar strains at 12 mg/kg/day (LOAEL).

Comments about Study and Endpoint: The rationale for not using the dermal developmental toxicity studies is presented in 2.3.2 Short-Term Subpopulation 13+. Since an oral dose was selected, a dermal absorption factor of 25.2% should be used in dermal risk assessments. Since an oral dose was selected, a dermal absorption factor of 25.2% should be used in dermal risk assessments.

This risk assessment is required.

2.3.5 Intermediate-Term Dermal - (7 days to Several Months) Infants and Children

Study Selected: Postnatal Developmental -Rat

MRID No. None. Presentation by Dr. Gray to SAP on 10/39/96

Executive Summary: See 2.3.3 Short-Term (Infants and Children)

Dose and Endpoint for Risk Assessment: NOAEL= 5 mg/kg/day based on delayed puberty at 15 mg/kg/day(LOAEL)**.**

Comments about Study and Endpoint: This endpoint is appropriate for use in risk assessment for this subpopulation (i.e., Infants and Children). This dose is supported by the NOAEL of 4.9 mg/kg/day established based on decrease in epididymal weights seen in male rats at 30 mg/kg/day in the two-generation reproduction study. Since an oral dose was selected, a dermal absorption factor of 25.2% should be used in dermal risk assessments.

This risk assessment is required.

2.3.6 Long-Term Dermal (Several Months to Life-Time)

Based on the use pattern, there are no Long-Term dermal exposure scenarios for the application of Vinclozolin and therefore there is minimal concern for potential exposure or risk. However, if a Long-Term exposure scenario is identified, then the MOEs should be calculated based on the following oral NOAELs and a 25.2% dermal absorption factor.

(I). Non-Cancer Effects

Study Selected: Chronic Toxicity -Rat §83-1a
Combined Chronic Toxicity - Rat §85-3

MRID Nos. 43254701, 43254702 and 43254703

Executive Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOAEL=1.2 mg/kg/day. based on foam cell aggregates in the lungs (males), eosinophilic foci in the liver (males), interstitial cell lipidosis in the ovaries (females) and lenticular degeneration of the eyes (both sexes) at 2.3 mg/kg/day (LOAEL).

Comments about Study and Endpoint: This oral NOAEL with a dermal absorption factor of 25.2% should be used **only for non-cancer** dermal risk assessments.

This risk assessment is required if Long-Term dermal exposures are identified.

(ii). Carcinogenic Effects

Study Selected: Two-Generation Reproduction -Rat §83-4

MRID Nos. 42581301 and 43254705

Executive Summary: In a two-generation reproduction study, Wistar rats were fed diets containing Vinclozolin at 0, 50, 300, 1000 or 3000 ppm (equivalent to 0, 4.9, 30, 96 or 290 mg/kg/day in males and 0, 5.3, 31, 101 or 290 mg/kg/day for females) through the PO, F_1 and F_2 generations for 14 weeks. Two litters per generation were produced. Two litters per generation were produced: F1a (F1 adults), F_{1b} (FX adults), F_{2a} (FY adults) and F_{2b} (FZ adults). FY and FZ adults were dosed only at 50 or 300 ppm because no F2 pups produced at higher dose levels. For parental systemic toxicity, the NOAEL was 4.9 mg/kg/day and the LOAEL was 30 mg/kg/day based on decreases in epididymal weights in males and possibly increases in liver weights. For offspring toxicity, the NOAEL was 4.9 mg/kg/day and the LOAEL was 30 mg/kg/day based on decreases in epididymal weights in the F_1, FX, FY and FZ males at 30 mg/kg/day.

Dose and Endpoint for Risk Assessment: NOAEL=4.9 mg/kg/day based on decreases in epididymal weights at 30 mg/kg/day (LOAEL).

Comments about Study and Endpoint: Vinclozolin requires two types of cancer risk calculations.
(1) Vinclozolin is classified as a **Group C Carcinogen** based on statistically significant increase in Leydig cell tumors in rats with a non-linear approach (MOE) for carcinogenic risk assessments. The oral NOAEL of 4.9 mg/kg/day and appropriate dermal (25.2%) and inhalation (100%) absorption factors should be used **carcinogenic** risk assessments.
(2) In addition a default Q* (mg/kg/day) $^{-1}$ of 2.9 x 10 $^{-1}$ was assessed for vinclozolin (Memorandum from Lori L Brunsman to David Anderson (10/21/98) Vinclozolin Quantitative Risk Assessment (Q*) Based on Wistar (Chbb:THOM SPF). Rat Chronic and Oncogenic Dietary Studies With 3/4 ‘s Interspecies Scaling Factor.)(Memorandum reproduced in the Appendix) Since an appropriate MOE has not been determined for Leydig cell tumors acting through a hormonal mechanism, a risk assessment using the Q* of 2.9 x 10 $^{-1}$ should also be conducted for vinclozolin.

NOTE: Non-cancer and/or carcinogenic risks are required ONLY if Long-Term exposure scenarios are identified.

2.3.7 Inhalation Exposure (Any-Time period)

Except for an acute inhalation toxicity study, the results of which place Vinclozolin in Toxicity Category IV (LC_{50} = 29.1 mg/L), no other studies are available via this route. Therefore, the HIARC selected the oral dose for inhalation risk assessments and should follow the route-to-route extrapolation as follows:

Step I. The inhalation exposure component (i.e., ：g a.i/lb/day) using a 100% absorption rate (default value) should be *converted to* an *equivalent oral dose* (mg/kg/day).

Step II. The dermal exposure component (i.e., mg/kg/day) using a 25.2% dermal absorption rate should be *converted to an equivalent oral dose*. This dose should then be combined with the converted oral dose in Step I.

Step III The combined dose from Step II should then be *compared to the oral doses* to calculate the MOE's.

For Short-and Intermediate-Term exposures, the oral doses are 3 mg/kg/day for Females 13 + and 5 mg/kg/day for Infants and Children.

The use pattern indicates no Long-Term inhalation exposure concern. However, if a scenario is identified, then the MOEs for Long-Term exposures should be based on the oral NOAELs of 1.2 mg/kg/day for Chronic effects and 4.9 mg/kg/day for carcinogenic effects.

Short-and Intermediate-Term inhalation exposure risk assessments are required. Long-Term exposure risk assessment is required ONLY if a Long-Term exposure scenario is identified for this route.

2.3.8 Margin of Exposure for Occupational/Residential Exposures:

An MOE of 100 is appropriate for occupational exposure assessments. The MOEs for residential exposure assessments will be determined by the FQPA Safety Factor Committee.

3.0. RECOMMENDATIONS FOR AGGREGATE EXPOSURE

3.1 For acute aggregate exposure, combine the high end exposure values for food plus water and compare to the acute RfD.

3.2 For Short-Term and Intermediate-Term aggregate exposure risk assessment, oral, dermal and inhalation exposures are combined, i.e., added when oral, dermal and inhalation are converted to the oral equivalent doses.

3.3 For Long-Term aggregate exposure risk assessment is needed only if there is a potential log term exposure (dermal and inhalation) concern. The pathways can be combined because of the use of oral equivalent doses. The Long-Term oral exposure can not be combined due to a different endpoint. In this case the MOEs for oral, dermal and inhalation can be combined

through the reciprocal equation to calculate at total MOE.

4.0. FQPA CONSIDERATIONS

1. Neurotoxicity Data Based on a review of 89 published literature papers and reviews, antiandrogens were shown to cause effects in the brains of developing treated rats (for summarized details, please see attachment, "Memorandum, dated 10/27/99, from Elizabeth Mendez to Jess Rowland and Pauline Wagner, Co-Chairs of the HIARC). At the *in vivo* level, exposure to Vinclozolin at a dose of 6.25 mg/kg/day results in significant anatomical abnormalities in males including reduced ano-genital distance (AGD), retained nipples, areolas, and reduced prostrate weight. In the developing brain the effects of androgenic-steroid exposure are more subtle, but profoundly influence the sex differentiating programs in the developing brain in frogs, birds, rats, mice, gerbils, guinea pigs, and monkeys. Although Gray and Ostby, 1998, [Gray, LE and Ostby, J (1998) Toxicology and Industrial Health *14 (No.½)*: 159-184] found no effects on rough and tumble play and mating behavior at 200 mg/kg/day, the studies may not have used sufficient animals to show a meaningful NOAEL.

Also considered were that no acute or subchronic neurotoxicity studies on Vinclozolin are available, but in a complete battery of subchronic and chronic studies and a reproduction study, as required for a food-use chemical, there were no indications of treatment-related effects on the central or peripheral nervous system of mice, rats, or rabbits. No changes in clinical signs, brain weights, gross necropsy results or histopathological results suggested any part of the nervous system as a target organ. In addition, no effects were seen in some developmental parameters/endpoints evaluated in the reproduction toxicity study such as pinna unfolding, auditory canal opening, eye opening, pupil constriction and gripping reflex.

2. Determination of Susceptibility

The developmental toxicity of Vinclozolin has been tested: 1) following oral administration in Long Evans Hooded and Wistar rats, and New Zealand white rabbits; and 2) following dermal application in Wistar rats. Reproductive toxicity was assessed in Wistar rats.

Prenatal studies in rats demonstrated enhanced susceptibility of rat fetuses as compared to maternal animals following *in utero* exposures. There was no indication of additional susceptibility to young rabbits following *in utero* exposures or in the prenatal dermal developmental toxicity studies in rats. In the two generation reproduction study in rats, effects in the offspring were observed only at, or at higher doses than those that caused parental toxicity.

In the oral prenatal developmental toxicity studies in rats, increased susceptibility was manifested as decreased ano-genital distance (AGD) in male fetuses at 12 mg/kg/day. This appeared to be the most sensitive indicator of Vinclozolin-induced toxicity. Alone, decreased AGD is of uncertain concern for human risk assessment, but when considered with effects occurring at higher dose, decreased AGD is a sensitive indicator for anti-androgenic effects of

Vinclozolin. However, there is suggestive evidence that the AGD in male rats may not be a permanent effect at low doses (≤ 50 mg/kg/day).

Also seen in rat fetuses following oral administration were increase in areolas/nipple anlagen in male fetuses at 12 mg/kg/day in two strains of rats, in Long-Evans Hooded and Wistar rats. In one study, permanent nipple development in male fetuses was reported at 3 mg/kg/day, however, the HIARC had low confidence the areolas development at 3 mg/kg/day and in the nipple development in 1 pup at 3 mg/kg/day.

The available data on mode of action demonstrated that the antiandrogenic properties of Vinclozolin is consistent with the developmental effects observed. Vinclozolin metabolites are competitive antagonists at the androgen receptor.

3 Results of the Developmental and Reproductive Toxicity Studies:

The results of the developmental studies (5 oral and 1 dermal) and the single reproductive toxicity studies are described in detail in the Developmental and Reproductive Toxicity Peer Review Committee Report of November 8, 1994. Results of the newly (1997) submitted data that were used in toxicology endpoint selection for the various exposure scenarios are presented earlier in this report.

HED's Reproductive and Developmental Toxicity Assessment Committee evaluated the newly available data and previously considered data in a weight-of-the-evidence approach. The Committee's findings are presented below:

A. Numerous effects in **rat** developmental and reproductive studies demonstrated the anti-androgenic effects of Vinclozolin at various dose levels as described below:

B. Decreased ventral prostate weight in males offspring rats occurred as low **6.25 mg/kg/day** with a NOAEL of 3.125 mg/kg/day. This effect also occurred in dogs, but the relevance to humans in not known. When this effect is taken into consideration with other effects occurring at higher dose levels, it provides a sensitive indicator for anti-androgenic effects and it is a physiological response to in utero exposure to vinclozolin.

C. There was consideration of a lower NOAEL based on possibly decreased AGD and areolas/nipple development at 3.125 mg/kg/day doses in the Gray study. Potential effects at the 3.125 mg/kg/day dose level were not selected because:

- The decrease in AGD in the Gray study was statistically significant (using multiple comparison tests) at 12 mg/kg/day and above.
- There was wide variation for AGD in the different control groups in the Gray study and a larger number of litters in each block in the BASF study.
- There was no indication that the nipple development in 1 pup at 3.125 mg/kg/day was histologically verified.

D. Also supporting the evidence for an anti-androgenic effect at 12 mg/kg/day was an increased number of "areolas" (BASF studies) in male rats. Since these areas were not examined histologically, they should be described as "hairless areas" until confirming histopathology has been performed, although they do provide supportive evidence for an anti-androgenic effect at 12 mg/kg/day in the BASF studies.

- Gray et al., 1999 reported an increased number of "nipples" (statistically significant) at 50 and 100 mg/kg/day and non- statistically significant incidences of nipples at 3.125, 6.25, 12.5, 25 mg/kg/day. Although these animals received a necropsy, histological evidence of nipple formation was confirmed only at 12.5 mg/kg/day.

- Gray, 1996 and Gray et al 1999 also reported a dose-related increase in "nipple areas" in 13-day old pups in all treatment groups. These observations were not supported by histological examination.

- The reports of "nipples", "nipple areas", and "areolas" give support for a NOAEL of 6.25 mg/kg/day based on decreased AGD and the NOAEL of 3.125 mg/kg/day for prostate weight, although histological confirmation, expected from both laboratories, will provide stronger evidence.

E. In general, the dose-response curve was shallow. Following are the main developmental effects reported in the developmental and reproductive studies:

- At **6.25 mg/kg/day** statistically significantly decreased prostate weights from perinatal studies were seen. This effect was supported by areolas and nipple development in the study.

- At **15 mg/kg/day**, delayed puberty was seen in a chronic study.

- Occurring at **15 mg/kg/day** and above in male rats was an increase in serum luteinizing hormone in a chronic study.

- At **25-31 mg/kg/day** and above in male rats, variations in parental and/or offspring organ weights (adrenal, epididymis, liver, testis, seminal vesicles, and ventral prostate weight) occurred.

- At **50 mg/kg/day** and above in male rats, an increase in hypospadia and a decreased sperm count in offspring occurred.

- At **101 mg/kg/day** and above, offspring in the rat reproduction study sired no pups due to functional malformations in males. Abnormalities in males included pseudohermaphroditism, atrophic seminiferous tubules, reduced penis size, aberrant Wolffian duct, bilateral Muellerian duct, and reduced or absent prostate, seminal vesicle, or bulbourethral gland. In female rats, abnormalities included ovarian lipidosis and ovarian interstitial cell hypertrophy.

- At **300-400 mg/kg/day**, dilated renal pelvis, dilated ureter, hydroureter, hydronephrosis, 14th rib, and delays in maturation also occurred.

- In the **rabbit** oral developmental study, developmental effects seen at **400 mg/kg/day** and above included an increase in early resorptions with a resulting decreased litter size, and skeletal anomalies. At **800 mg/kg/day**, abortions also occurred. No anti-androgenic effects were noted.

- In the dermal prenatal developmental studies, for maternal toxicity, the NOAEL was 60 mg/kg/day and the LOAEL was 180 mg/kg/day based on increased absolute adrenal weights. For developmental toxicity, the NOAEL was 60 mg/kg/day and **the LOAEL was 180 mg/kg/day** based on statistically significantly decreased AGD in male fetuses. At 360 mg/kg/day, nominally increased incidence of dilated renal pelvis and hydroureter was reported. Dilated renal pelvis incidence was 88% in litters vs 83% in historical controls for the incidence of dilated renal pelvis and hydroureter combined.

F. The developmental and reproductive effects were evident in the rat studies, with no anti-androgenic effects in the rabbit developmental study. However, the mechanism for anti-androgenicity is believed to be antagonistic binding to the androgen receptor by Vinclozolin metabolites. Since the androgen receptor is widely conserved across species lines, anti-androgenic effects would be expected in humans. In addition, in the chronic dog study, increased relative testes weight, decreased prostate weight, and prostate atrophy occurred.

The Committee conclude that with the above considerations, and careful evaluation of all the data submitted regarding developmental and reproductive toxicity of Vinclozolin, the NOAEL for developmental toxicity was 3 mg/kg/day with a LOAEL for developmental toxicity of 6 mg/kg/day.

4. Recommendation for Developmental Neurotoxicity Study

The Committee determined that, based on a weight-of-the-evidence after reviewing the literature available (See the attachment), that a developmental neurotoxicity study in rats with Vinclozolin is required. The committee acknowledges that the current DNT protocol may not be sufficient to detect the subtle findings reported in the current literature, and an expanded DNT study protocol may be necessary to assess the relevant endpoints. The committee also recommended that an *ad hoc* committee be convened to determine the most appropriate endpoints and procedures to assess vinclozolin's potential to affect changes in the development of the nervous system. The following information was considered in arriving at this decision.

- A report by Wong *et al.*[1] indicates that vinclozolin, M1, and M2 at concentrations ranging from 0.5 to 20 µM can inhibit binding of a synthetic androgen (R1881) to the AR. Furthermore, transcription of androgen-regulated genes is inhibited by as much as 80% after exposure to 0.2 µM of M2 .
- Androgenic-steroid exposure profoundly influence sex differentiating programs in the developing brain in frogs, birds, rats, mice, gerbils, guinea pigs, and monkeys.[2]
- A 1977 report by Lieberburg *et al.* provides strong evidence to support this statement by showing that treatment with cyproterone acetate (an anti-androgen) significantly inhibits dihydrotestosterone (DHT) binding to the AR receptor in the pituitary as well as the limbic brain (preoptic area , septum, hypothalamus, and amygdala). Also noteworthy is that the highest concentration of AR-binding takes place in the limbic brain which has been implicated in "characteristic male behavior such as aggression" as well as motivational function.[3]
- Male rhesus monkeys display an earlier development in the ability to perform object discrimination reversal (a behavioral test; see table for brief description). Androgenized female fetuses or newborn infant fetuses behave in a manner similar to males. Conversely, castrated males performed better in a visual discrimination task - in which females usually perform better than males - when

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[1] Wong, C. *et al. Androgen Receptor Antagonist versus Agonist Activities of the Fungicide Vinclozolin Relative to Hydroxyflutamide* (1995) **The Journal of Biological Chemistry 270 (34) pp. 19998-20003.**

[2] Cooke, B. *et al. Sexual Differentiation of the Vertebrate Brain: Principles and Mechanisms* (1998) **Frontiers in Neuroendocrinology 19 pp. 323-362**

[3] Lieberburg, I. *et al. 5% Dihydrotestosterone Receptors in Rat Brain and Pituitary Cell Nuclei* (1977) **Endocrinology 100 pp. 598-607.**

compared to their intact counterparts and to a level comparable to females.[4]

- **P** The effects of anti-androgenic compounds on the nervous system are not necessarily limited to the brain but may affect the peripheral nervous system as suggested by research conducted by Cooke *et al.*[2]
- **P** The importance of aromatase function in the development of the brain is further suggested in studies by Beyer *et al.*[5] Testosterone treatment of embryonic hypothalamic regions of the brain (note that this is part of the limbic brain) resulted in an increase in the soma size, total neurite length, and number of stem processes in aromatase-immunoreactive [AR-IR] (i.e. containing high levels of the aromatase enzyme) neurons.
- **P** An adequate study showing a NOAEL for CNS effects has not been conducted.

Other information also considered in the weight of evidence.

- **#** No evidence of abnormalities in the development of the fetal nervous system, were observed in the prenatal developmental toxicity studies in two strains of rats and rabbits following oral exposure and in one strain of rats after dermal exposure at maternally toxic doses.

- **#** No clinical evidence of behavioral alterations was observed in pups from the two-generation reproduction study in rats in BASF studies.

- **#** No effects were seen in some developmental parameters/endpoints evaluated in the reproduction toxicity study such as pinna unfolding, auditory canal opening, eye opening, pupil constriction and gripping reflex.

- **#** Neither brain weight nor histopathology (nonperfused) of the nervous system were affected by treatment in subchronic and chronic toxicity studies in mice, rats or dogs.

- **P** Gray and Ostby [6] showed that rough and tumble play and mounting behavior

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[4] Bachevalier, J. and Hagger, C. *Sex Differences in the Development of Learning Abilities in Primates* (1991) **Psychoneuroendocrinology 16 (1-3) pp. 177-188.**

[5] Beyer, C. and Hutchison, J.B. *Androgens Stimulate the Morphological Maturation of Embryonic Hypothalamic Aromatase-immunoreactive Neurons in the Mouse* (1997). **Developmental Brain Research 98 pp. 74-81.**

[6] Gray, L.E. and Ostby, J. (1998) *Effects of pesticides and toxic substances on behavioral and morphological reproductive development: Endocrine versis nonendocrine mechanisms.* **Toxicology and Industrial Health *14 (No. ½)*: 159-184.**

were not affected in perinatal studies with vinclozolin at high dose levels causing hypospadia.

- The literature shows CNS effects from antiandrogens, but generally at dose levels causing malformations of the reproductive organs. However, the studies cited were conducted a high dose levels showing publishable effects and very few studies of the CNS were done at low dose levels showing a NOAEL.

- Although not specifically studied, the literature studies reviewed shows no CNS effects from androgen deprivation at low dose levels below those causing AGD decreases or male organ weight decrement.

5. Hazard-based FQPA Safety Factor Recommendation:

Based on hazard alone, the HIARC recommended that the FQPA Safety Factor should be retained. The final safety factor recommendation will be made by the FQPA Safety Factor Committee.

5.0 DATA GAPS

Developmental Neurotoxicity Study.

6.0 ACUTE TOXICITY

ACUTE TOXICITY VALUES - VINCLOZOLIN

Test	Result	Toxicity Category
Acute Oral LD50 in Rats MRID# 00080451 & 92194010, Study# BASF XXII/337, 90/6515, Date 2/20/73.	LD50 > 10,000 mg/kg in both males and females. Acceptable	IV
Acute Dermal LD50 Rats MRID# 00086339 & 921934011, Study# BASF 90/6516, Date 11/2/77.	LD50 > 2500 mg/kg in both males and females. Acceptable	III
Acute Inhalation LC50 in rats MRID# 00075474 & 92194012, Study# 90/6517, Date 4/20/79.	LC50 > 29.1 mg/l. Acceptable	IV
Primary Eye Irritation in Rabbits MRID# 00086341 & 92194013, Study# BASF 90/6518, Date 11/9/77	Slight eye irritation cleared by day 8. Acceptable	III
Primary Skin Irritation in Rabbits MRID# 00086340 & 92194014, Study# BASF 90/6519, Date 11/9/77.	Slight skin irritation cleared within 72 hours. Acceptable	IV
Skin Sensitization in Guinea Pigs MRID# 00080451 & 92194015, Study# BASF 90/6520, Date 9/7/79.	Skin sensitizer in 4/9 GP. Acceptable	Sensitizer

7.0 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Females 13+)	Adjusted Developmental NOAEL=6	Decreased ventral probate weights.	Developmental- Rat
	Acute RfD = 0.06 mg/kg/day		
Acute Dietary (Infants & Children)	No appropriate endpoint was identified in oral toxicity studies including the developmental and reproductive toxicity studies in rats and rabbits for risk assessment for this subpopulation.		
Chronic Dietary	NOAEL=1.2	Histopathological lesions in the in the lungs (males), liver (males), ovaries (females) and eyes (both sexes)	Combined Chronic Toxicity/Carcinogenicity- Rat
	General population Chronic RfD = 0.012 mg/kg/day		
Carcinogenic Dietary	NOAEL=4.9	Vinclozolin is classified as a Group C carcinogen with a non-linear (MOE) approach	2-Generation Reproduction- Rat
Carcinogenic Dietary	Q* = 2.9×10^{-1} $(mg/kg/day)^{-1}$	Vinclozolin is classified as a Group C carcinogen for Testicular Leydig cell tumors with a Q* $(mg/kg/day)^{-1}$ of 2.9×10^{-1} in human equivalents.	Combined Chronic Toxicity/Carcinogenicity- Rat
Chronic dietary risk should be conducted by two approaches (1) the MOE, and (2) the Q* approach			
Short-& Intermediate Term (Dermal) [a] Females 13 +	Oral NOAEL=3	Decreased ventral prostate weights	Developmental -Rat
Short-& Intermediate Term (Dermal) [a] Infants & Children	Oral NOAEL = 5	Increased incidence of delayed puberty	Developmental -Rat
Long-Term (Dermal) [a] **Non-Cancer**	Oral NOAEL = 1.2	**Risk assessment is required only if a scenario is identified** for this exposure. A 25.2% dermal absorption factor should be used for non-cancer dermal risk assessment.	

Long-Term (Dermal) [a] **Cancer**	Oral NOEL = 4.9 mg/kg/day and Q* = 2.9×10^{-1} $(mg/kg/day)^{-1}$	Classified as a **Group C carcinogen** with a **non-linear (MOE) approach. Risk assessment is required only if a scenario is identified**. A 25.2% dermal absorption factor of 25.2% should be used for carcinogenic dermal risk assessment. Also use the Q* in a low dose extrapolation for human risk assessment where appropriate.	
*Inhalation (Short-& Intermediate-Term) [a] Females 13 +	Oral NOAEL = 3 mg/kg/day	Decreased ventral prostate weights	Developmental -Rat
*Inhalation (Short-& Intermediate Term) [a] Infants & Children	Oral NOAEL = 5 mg/kg/day	Increased incidence of delayed puberty	Developmental -Rat
Inhalation (Long-Term) [a] All subpopulation	Oral NOEL = 1.2	**Risk assessment is required only if a scenario is identified**. A 100% (default value) inhalation absorption factor should be used for this risk assessment.	

a = Appropriate route-to-route extrapolation should be performed for these risk assessments (i.e., the dermal inhalation exposure components using the appropriate absorption rates (25.2% for dermal and 100% for inhalation) should be converted to equivalent oral doses and compared to the oral NOEL.

*Revised to correct error (separate endpoints for Females 13+ and Infants/Children as specified in Section 2.3.7 of this report); 01/05/00; BSTarplee

6.0 ATTACHMENT 1: Memorandum, dated 10/27/99 from Elizabeth Mendez of HED to Jess Rowland and Pauline Wagner, Co-Chairs of HIARC.

6.0 ATTACHMENT 2: Memorandum from Lori L Brunsman to David Anderson (10/21/98) Vinclozolin Quantitative Risk Assessment (Q*) Based on Wistar (Chbb:THOM SPF). Rat Chronic and Oncogenic Dietary Studies With 3/4 's Interspecies Scaling Factor.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: October 27, 1999

MEMORANDUM

SUBJECT: VINCLOZOLIN: Evaluation of the need to request a Developmental Neurotoxicity Study
PC Code: 113201

TO: Jess Rowland, Branch Chief - Reregistration Branch III
Co-Chairperson
Hazard Identification Assessment Review Committee

and

Pauline Wagner, Branch Chief - Reregistration Branch II
Co-Chairperson
Hazard Identification Assessment Review Committee

FROM: Elizabeth Méndez, Ph.D.
Toxicologist
Reregistration Branch I/HED (7509C)

THROUGH: Whang Phang, Ph.D.
Branch Senior Scientist
Reregistration Branch I/HED (7509C)

On August 31, 1999 the Health Effects Division Hazard Identification Assessment Review Committee (HIARC) re-evaluated the toxicology database of Vinclozolin and re-assessed the Reference Dose (RfD) and toxicological endpoints selected for acute dietary exposure. The HIARC also determined that a comprehensive evaluation of the open literature on Vinclozolin was necessary to consider the potential developmental neurotoxicity effects and the need to request a Developmental Neurotoxicity Study as part of the Vinclozolin database. An evaluation of the currently available literature that related to androgenic activity has been conducted. For this analysis a total of 89 published articles (including reviews and research articles) were evaluated. This memorandum summarizes the relevant findings of this evaluation. In addition, the attachment

(Table[7]) summarizes the relevant individual reports and the interpretation of this reviewer.

A thorough review of the open literature on Vinclozolin indicates that this chemical and its metabolites - M1 and M2 - have anti-androgenic properties and can effectively compete for androgen receptor (AR) binding with both synthetic (R1881) and naturally occurring androgens (testosterone). A report by Wong *et al.*[8] indicates that vinclozolin, M1, and M2 at concentrations ranging from 0.5 to 20 µM can inhibit binding of a synthetic androgen (R1881) to the AR. Furthermore, transcription of androgen-regulated genes is inhibited by as much as 80% after exposure to 0.2 µM of M2 . This finding is consistent with data obtained in mobility shift assays where exposure to 0.2 - 0.5 µM (57.2 µg - 143 µg) of M2 resulted in complete inhibition of AR binding to the androgen-response-element (ARE) in the promoter region of the afore mentioned genes. Evaluated in conjunction with immunochemistry data in which exposure to 0.5 µM M2 yielded significant AR translocation from the cytoplasm to the nucleus, these findings strongly suggests that this chemical exerts its effect through binding to the AR.

At the *in vivo* level, exposure to Vinclozolin at a dose of 3.125 mg/kg/day results in significant anatomical abnormalities in males including but not limited to reduced ano-genital distance (AGD), retained nipples, areolas, and reduced prostrate weight. In the developing brain the effects of androgenic-steroid exposure are more subtle, but profoundly influence the sex differentiating programs in the developing brain in frogs, birds, rats, mice, gerbils, guinea pigs, and monkeys.[9] As a result, any chemical that interferes with the function of an androgenic- steroid (via anti-androgenic activity) has the potential of significantly disrupting the neuroendocrine system. A 1977 report by Lieberburg *et al.* provides strong evidence to support this statement by showing that treatment with cyproterone acetate (an anti-androgen) significantly inhibits dihydrotestosterone (DHT) binding to the AR receptor in the pituitary as well as the limbic brain (preoptic area , septum, hypothalamus, and amygdala) Since cyproterone acetate has been demonstrated to interact with the AR in a manner similar to vinclozolin and its metabolites, the possibility exists that vinclozolin, M1, and M2 may have similar if not identical effects on the brain. Also noteworthy is that the highest concentration of AR-binding takes place in the limbic brain which has been implicated in "characteristic male behavior such as aggression" as well as motivational function.[10]

The correlation between AR binding/activation of androgen-regulated genes and the limbic brain is a recurrent observation noted in several papers in the open literature. The effects of anti-androgenic compounds on the nervous system are not necessarily limited to the brain but may affect the peripheral nervous system as suggested by research conducted by Cooke *et al.*[2] In this series of experiments, insensitivity to steroids (or treatment with anti-androgens) leads to the feminization of the Spinal Nucleus Bulbocavernosus (SNB) System

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[7] An electronic copy is unavailable, but a copy is available in the file.

[8] Wong, C. *et al. Androgen Receptor Antagonist versus Agonist Activities of the Fungicide Vinclozolin Relative to Hydroxyflutamide* (1995) **The Journal of Biological Chemistry 270 (34) pp. 19998-20003.**

[9] Cooke, B. *et al. Sexual Differentiation of the Vertebrate Brain: Principles and Mechanisms* (1998) **Frontiers in Neuroendocrinology 19 pp. 323-362**

[10] Lieberburg, I. *et al. 5% Dihydrotestosterone Receptors in Rat Brain and Pituitary Cell Nuclei* (1977) **Endocrinology 100 pp. 598-607.**

[this system innervates the bulbocavernosus (BC) and levator ani (LA) muscles attached to the penis] by inhibiting the innervation of the BC muscles and reducing the number of SNB neurons. This suggests that some of the sexual behavioral deficits observed in anti-androgen treated animals may not only be due to anatomical abnormalities but also due to inadequate innervation of the reproductive organs. In a parallel experiment perinatal androgen exposure of females (XX) prevents the developmental apoptosis of the SNB system (i.e the urogenital region is innervated in the same manner as "normal XY" males).

Effects of either castration or anti-androgen treatment on brain development have also been reported in zebra finches, japanese quails, and rhesus monkeys. In zebra finches, anti-androgen exposure of juveniles led to an impairment in their song learning behavior which is a crucial component of the courtship/mating behavior. Noticeably, this effect is only seen in birds treated within a "narrow time-frame" during development since similar treatment to adults did not result in changes in the song pattern. The effects on the reproductive behavior of the Japanese Quail included a decrease incidence of strutting and head-grabbing behavior (typical male courtship rituals). Male rhesus monkeys display an earlier development in the ability to perform object discrimination reversal (a behavioral test; see table for brief description). Androgenized female fetuses or newborn infant fetuses behave in a manner similar to males. Conversely, castrated males performed better in a visual discrimination task - in which females usually perform better than males - when compared to their intact counterparts and to a level comparable to females.[11]

At the molecular level, testosterone was shown to be converted into 17$-estradiol in the brain by an enzyme called aromatase. Aromatization of testosterone to 17$-estradiol is a required intermediate step in the masculinization of the brain. This observation is supported by various lines of evidence including:

1) treatment of animals with estradiol benzoate are more efficiently masculinized than animals treated with testosterone propionate.
2) reduced metabolites (such as DHT) which cannot be aromatized are less effective at masculinizing animals than their aromatizable counterparts (testosterone).
3) aromatase inhibitors block brain masculinization similar to the effects seen after anti-androgen treatment.
4) anti-androgen treatment blocks testosterone-induced aromatase transcription.

The importance of aromatase function in the development of the brain is further suggested in studies by Beyer *et al.*[12] Testosterone treatment of embryonic hypothalamic regions of the brain (note that this is part of the limbic brain) resulted in an increase in the soma size, total neurite length, and number of stem processes in aromatase-immunoreactive [AR-IR] (i.e. containing high levels of the aromatase enzyme) neurons. Neurite branching and the absolute number of AR-IR neurons in the hypothalamic cultures were also dramatically increased . Administration of the anti-androgen flutamide abolished the testosterone-induced morphological changes. That the aromatase-inhibiting properties of this compound are the main causes of this phenotype is suggested by the

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[11] Bachevalier, J. and Hagger, C. *Sex Differences in the Development of Learning Abilities in Primates* (1991) **Psychoneuroendocrinology 16 (1-3) pp. 177-188.**

[12] Beyer, C. and Hutchison, J.B. *Androgens Stimulate the Morphological Maturation of Embryonic Hypothalamic Aromatase-immunoreactive Neurons in the Mouse* (1997). **Developmental Brain Research 98 pp. 74-81.**

fact treatment with an anti-estrogen (tamoxifen) did not result in the changes in morphology indicating that the conversion of testosterone to estradiol is a crucial step in the morphological changes observed in the hypothalamus.

Perhaps the most striking example of the effect that androgen and consequently anti-androgen activity has on the developing brain is provided by human case studies. Female patients with Congenital Adrenal Hyperplasia (CAH) - who are exposed to excessive levels of androgens prenatally - are born with virilized genitalia that is surgically corrected shortly after birth. These patients are then immediately placed on hormone therapy. Nonetheless, these females display more rough-and-tumble play and prefer boys as play partners. At the other end of the spectrum, are patients born with 5"-reductase (necessary for reduction of testosterone to DHT) or 17$-hydroxylase (necessary for 17$-estradiol synthesis) deficiencies are unable to produce androgens necessary for masculinization of the external genitalia. As a result, they are assigned the female sex at birth and are raised as girls. During puberty, however, the external genitalia virilize and many of these individuals choose to live as males.

In conclusion, there is sufficient evidence that compounds like Vinclozolin that may disrupt the neuroendocrine system through their anti-androgenic properties can cause significant changes in the morphological and biochemical development of the nervous system. The effects of these changes can be monitored at the molecular level by use of immunocytochemistry, fluorescent *in situ* hybridization (FISH), mobility shift assays, or transcription assay techniques. At the organism level, behavioral tests can be conducted to ascertain the effects of anti-androgen treatment in learning and behavior. After an evaluation of the available literature, it is this reviewer's opinion that a request for the submission of a Developmental Neurotoxicity Study is warranted. This study, however, may need to be appropriately modified for assessing the potential effects of Vinclozolin in brain development.

HED DOC. NO. 012950

MEMORANDUM October 21, 1998

SUBJECT: Vinclozolin Quantitative Risk Assessment (Q_1^*) Based On Wistar (Chbb:THOM) (SPF)) Rat Chronic and Oncogenic Dietary Studies With $^3/_4$'s Interspecies Scaling Factor

P.C. Code 113201

TO: David Anderson, Toxicologist
Reregistration Branch 2
Health Effects Division (7509C)

AND: Michael Metzger, Branch Chief
Reregistration Branch 1
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

Summary

The following unit risk, Q_1^* $(mg/kg/day)^{-1}$, of Vinclozolin has been calculated at the request of Bill Burnam. It is not meant to imply that a risk assessment should use a Q_1^* $(mg/kg/day)^{-1}$, but is strictly for illustrative purposes. The unit risk has been converted from animals to humans by use of the $^3/_4$'s scaling factor (Tox_Risk program, Version 3.5, K. Crump, 1994)[13].

The unit risk, Q_1^* $(mg/kg/day)^{-1}$, of Vinclozolin based upon male rat testicular interstitial cell tumor rates is 2.9×10^{-1} in human equivalents. For purposes of this risk assessment, the oncogenic and chronic dietary studies have been combined. The dose levels used in this assessment were 0, 50, 150, 500, and 1500 ppm of Vinclozolin. The corresponding tumor rates for the male rat testicular interstitial cell tumors were 34/68, 25/49, 12/20, 64/70, and 19/20, respectively. The highest dose levels of both studies and their corresponding tumor rates

[13]See memo - Deriving Q_1^*s Using the Unified Interspecies Scaling Factor, P.A. Fenner-Crisp, Director, HED, 7/1/94.

were excluded from this analysis, as the cancer peer review commitee, at its January 15, 1997 meeting, decided that these dose levels were excessive .

Background

The statistical evaluation (Vinclozolin Qualitative Risk Assessment Based On Wistar (Chbb:THOM (SPF)) Rat and C57BL/6/JICO Mouse Dietary Studies, L. Brunsman, 7/10/95) indicated that there were no significant incremental changes in mortality with increasing doses of Vinclozolin in male rats.

The male rats had a significant dose-related increasing trend, and significant differences in the pair-wise comparisons of the 500 and 1500 ppm dose groups with the controls, for testicular interstitial cell tumors, all at $p < 0.01$.

Dose-Response Analysis

The estimate of unit risk, Q_1^*, was based upon testicular interstitial cell tumors observed in male rats.

The male rats had no statistically significant incremental changes in mortality with increasing doses of Vinclozolin, therefore, the estimate of unit risk, Q_1^*, was obtained by the application of the Multi-Stage model (Tox_Risk program, Version 3.5, K. Crump, 1994).

For the conversion to human equivalents, weights of 0.35 kg for the rat, 70 kg for humans and the $^3/_4$'s scaling factor were used.

It is to be noted that the Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper bound on risk and that, as stated in the EPA Risk Assessment Guidelines, “the true value of the risk is unknown, and may be as low as zero.”